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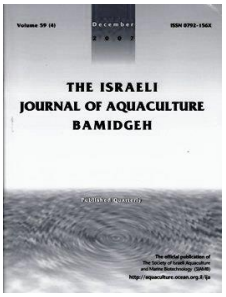

Triploidy Induction by Heat Shock in Mandarin Fish *Siniperca chuatsi*

Peng Xu[1,2], Shuang Zeng[1,2], Pengfei Wang[1,2], Qindong Tang[1,2], Lei Zeng[1,2], Lei Zhou[1,2], Guifeng Li[1,2]*

[1]*Institute of Aquatic Economic Animals, School of Life Sciences, Sun Yat-sen University, Guangzhou 510027, P.R. China*

[2]*South China Sea Bio-Resource Exploitation and Utilization Collaborative Innovation Center*

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Key words: triploidy induction；heat shock；mandarin fish； *Siniperca chuatsi*；

Abstract

Mandarin fish, *Siniperca chuatsi*, is an economically important fish due to its large size, fast growth, and delicious flesh. It is widely cultured in China. In this paper, triploidy in mandarin fish *S. chuatsi* was induced by heat shock. The most effective triploidy induction was achieved at 41°C, 8 min after fertilization for 2 min resulting in 40% triploid fish. There were no significant differences in the survival rates among the three treatment groups. Ploidy of fish was determined with a flow cytometer and chromosome counting. In conclusion, this paper presents optimal conditions for triploidy induction in mandarin fish with heat shock. The results will contribute to enhancement of its production in culture.

* Corresponding author. E-mail: liguif@mail.sysu.edu.cn

Introduction

Siniperca chuatsi (Mandarin fish), is an endemic freshwater fish species in eastern Asian countries (Froese and Pauly, 2014). It is an economically important fish due to its large size, fast growth, and delicious flesh. Mandarin fish culture is firmly established in China with a production of 0.28 million tons in 2012 (Bureau of Fisheries, Ministry of Agriculture of the People's Republic of China, 2013).

Sterile fish have been obtained in many species by chromosome set manipulation to induce triploidy (Cal et al., 2006; Derayat et al., 2013). Production of sterile fish is useful to the fish industry because sterility diverts energy from reproduction towards somatic growth, survival, and flesh quality (Maxime, 2008). One of the benefits of sterile fish is reduction of the risk of genetic interaction between farmed and wild stocks in the event of accidental or targeted introduction (Peruzzi et al., 2007).

Triploidization is commonly recognized as the most practical, economical, and effective method for large-scale production. Triploidy can be achieved by application of physical or chemical inductors during embryonic development that result in the retention of the second polar body (Maxime, 2008). The most common methods are thermal shock by heat or cold. Different factors such as temperature, duration, and initiation time of shock (Felip et al., 2001) can affect effectiveness in triploidy induction. In recent years, triploidy has been induced in many fish (Dube et al., 1991; Moffett and Crozier, 1995; Felip et al., 1997; El Gamal et al., 1999; Piferrer et al., 2000; Silva et al., 2007; Peruzzi et al., 2007; Kalbassi et al., 2009; Xu and Chen, 2010; Pradeep et al., 2014). In some fish species, triploids have a better growth rate than diploids (Qin et al., 1998; Cal et al., 2006; Xu et al., 2008). However, no information is available on the most suitable procedure for heat shock induction of triploid mandarin fish *S. chuatsi*.

Mass production of sterile individuals could significantly benefit production of mandarin fish *S. chuatsi* in aquaculture. The present study aims at investigating the optimal parameters for heat shock induction of triploid mandarin fish *S. chuatsi*. For this purpose, experiments were designed to test the treatment temperature, initiation, and duration time of heat shock that would result in a high proportion of triploidy in this species.

Materials and Methods

Broodstock management and gamete fertilization. The broodstock used in this study were obtained from BaiRong Aquatic breeding Co., Ltd (Guangdong, China). In May and June, during the spawning season, six males and six females were selected for artificial breeding. At the moment of artificial breeding, one female and one male were manually stripped to release eggs and sperm respectively. These were gently mixed and water was added to enable fertilization. The water temperature during fertilization and prior to the shock treatment was 25-26°C.

Induction of triploidy. Three treatments were designed. In each treatment, eggs and sperm from one female and one male were divided into approximately equal quantities and placed in individual 1000ml beakers.

Treatment 1 was divided into five sub-groups with different initiation times of thermal shocks of 2, 4, 6, 8, and 10 min after fertilization. The duration of the thermal shock for all the groups was 2 min and the applied temperature was 41^0C.

Treatment 2 was divided in three sub-groups all of which were exposed to, thermal shock of 41^0C. Initiation time of heat shock was 8 minutes after fertilization. Duration of the heat shock was 1, 2, and 3 minutes respectively.

Treatment 3 was divided in three sub-groups and exposed to a 2 min thermal shock with initiation time after fertilization of 8 min at different temperatures, 39^0C, 41^0C, and 43^0C respectively. (Table 1)

The control group comprised untreated samples of the same gametes and was considered as a normal diploid (2N) control in every treatment. The shock treatment on fertilized eggs collected from the same female was repeated three times for each set of experiments. The temperature of shock was maintained during the test period by a low temperature thermostat DC-5300. The untreated control group followed the same process.

Table 1. Treatments for heat shock triploidy induction *on S. Chutasi eggs* and their corresponding survival rates

Treatment	*Initiation time of heat shock (min after fertilization)*	*Shock duration (min)*	*Temperature (°C)（±0.05 °C）*	*Survival rates(%)*
1	2	2	41	*
	4			*
	6			*
	8			63.64±5.01
	10			*
2	8	1	41	86.73±1.99[a]
		2		83.83±5.83[a]
		3		*
3	8	2	39	60.20±11.39[b]
			41	57.90±6.61[b]
			43	*
Control	-	-	-	100

*=No survival.
a=No significant differences.
b=No significant differences.

Egg incubation and survival. Heat-shocked eggs were transferred to individual incubators (20L) for further incubation (3000 eggs per incubator). Survival rates were assessed 72h after fertilization and calculated as the number of live larvae in relation to the initial number of larvae.

Determination of ploidy. To determine ploidy, two-month-old mandarin fish *S. chuatsi* were sampled from the three treatment groups (Table 2). For analysis, ten μL of blood samples were mixed with 5 μL Permeabilization Solution (MultiSciences（Lianke）Biotech Co., Ltd.) and 50 μL DNA Prep Stain solution (Beckman Coulter, Inc.). The solution was incubated at room temperature for 20 min. The DNA content of approximately n=30 fishes per treatment was assessed with a Cytomics FC-500 (Beckman Coulter, Inc.) flow cytometer. Untreated diploid fish (n=5) were used as a standard to calibrate the flow cytometer.

Table 2. Triploidy rates in Mandarin fish *S. chuatsi* at different conditions

Treatment	*Initiation time of heat shock (min after fertilization)*	*Shock duration (min)*	*Temperature (°C)（±0.05 °C）*	*triploidy rates (%)*
1	8	2	39	10
2	8	1	41	6.67
3	8	2	41	40
Control	-	-	-	0

For more accurate analysis, the number of chromosomes were compared with those of nine-month-old diploid (n=3) and with triploid (n=3) mandarin fish whose ploidy was previously determined with a flow cytometer. The chromosome preparation method in this study was carried out according to Ojima and Kurishita (1980, with some modifications. At least thirty chromosome images per individual fish were counted to determine the ploidy.

Statistical analysis. The data were presented as means ± SD. Treatment effects were tested by ANOVA followed by Student's t-test using SPSS21.0 software. Differences were considered significant when $P<0.05$.

Results

The results are shown in Table 1 and Table 2. There were statistical differences between survival rates among treatments. In some treatments the larvae did not survive.

The most effective shock was 8 min after fertilization for 2 min, at 41°C. This treatment produced the highest triploidy rates (40%). In treatments 2 and 3, there were no significant differences in survival rates between the two treatment groups which produced triploids.

Distribution of DNA content are shown in Fig.1. Diploid mandarin fish *S. chuatsi* had a mean DNA content of 39.78 ± 2.56 (n = 5) and the triploid 60.02 ± 2.49 (n = 17).

The number of chromosomes is shown in Fig. 2. The diploid mandarin fish *S. chuatsi* had 48 chromosomes and the triploid mandarin fish *S. chuatsi* had 72 chromosomes.

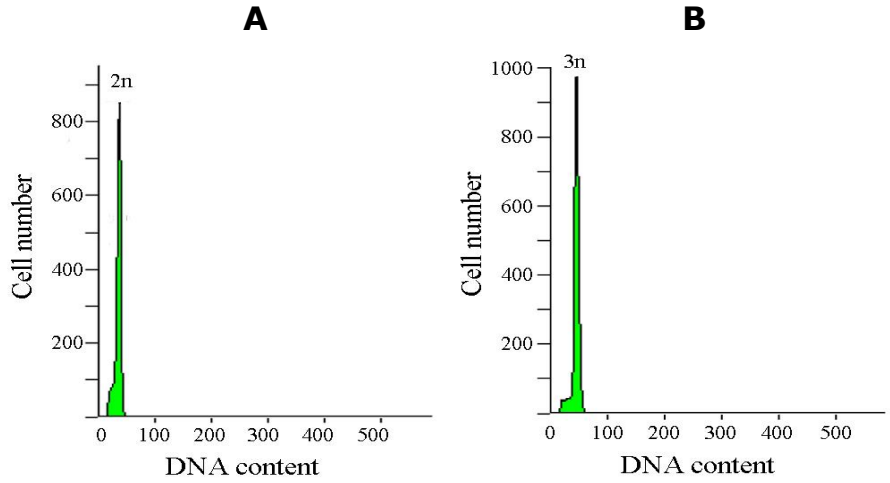

Fig. 1. DNA content of (A) diploid (control) and (B) heat-shocked triploid Mandarin fish *S. chuatsi*.

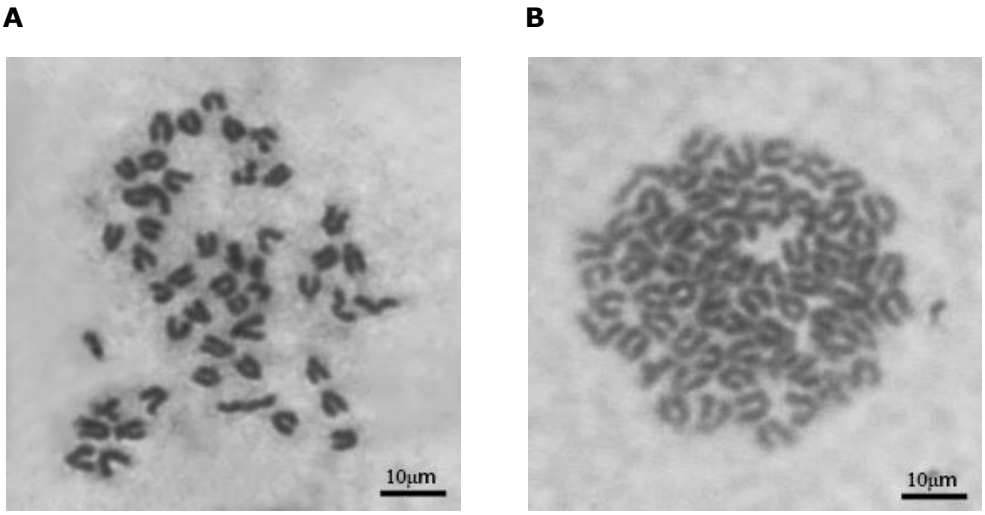

Fig. 2. Chromosomal numbers of (A) diploid (control) and (B)heat-shocked triploid Mandarin fish *S. chuatsi*.

Discussion

The present study reports induction of the triploidy in mandarin fish, *S. chuatsi*. and demonstrates how different shock conditions had different effects on survival rates. Survival was nil in treatment 1 after 2, 4, 6, and 10 minute shock at 41°C, but was 63% after the 8 minute treatment. This may indicate that the extrusion of the second polar body occurs at 8 min after fertilization in *S. chuatsi*. It is unlike Caspian salmon, *Salmo trutta caspius* (Kalbassi et al., 2009) which probably have two consecutive events that happen during meiosis, including spindle formation and absorbance of polar body into the ooplasm (Pandian and Koteeswaran, 1998). No individuals survived using heat shocks at higher temperatures (43 °C) or longer shock duration (3 min). Similar trends were also

reported in the triploidy induction by heat shock in brook trout *Salvelinus fontinalis* (Dube et al., 1991) and Caspian salmon, *Salmo trutta caspius* (Kalbassi et al., 2009).

In treatment 2, with the initiation time of the thermal shock of 8 min. after fertilization at 41°C, survival rates were 83% while under the same conditions for treatments 1 and 3, only 63% and 57% respectively, survived. There were some differences in survival rates among different females that underwent the same heat shock. The results indicated that survival rates by heat shock may be affected by other factors such as egg quality, degree of maturation of females, and differential susceptibility of egg batches (Felip et al. 2001; Peruzzi.et al. 2007).

In summary, the most effective conditions for triploidy induction in this study involved a 2 min heat shock of 41°C with induction time of 8 min after fertilization. This treatment produced the highest triploidy rates (40%). Lower heat shock temperatures or shorter shock duration had no significant differences on the survival rates but decreased the number of triploids. This paper provides a method for the mass production of triploid mandarin fish *S. chuatsi* and may enhance aquaculture production of this species.

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